09/980824 .031402

JC10 Rec'd PCT/PTO 07 DEC 2001

FORM PTO-1390 (Modified) (REV 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		016915-0252
		U S APPLICATION NO. (If known, see 37 CFR 1.5) Unassigned 09/980824
INTERNATIONAL APPLICATION NO. PCT/EP00/04848	INTERNATIONAL FILING DATE May 27, 2000	PRIORITY DATE CLAIMED June 7, 1999

TITLE OF INVENTION
USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON β-GLUCURONIDASE IN HUMAN TISSUE

APPLICANT(S) FOR DO/EO/US
Gerd GEISSLINGER, Heyo K. KROEMER, and Bernhard SPERKER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - ☒ has been transmitted by the International Bureau.
   - ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - ☐ have been transmitted by the International Bureau.
   - ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
11. ☒ Applicant claims small entity status under 37 CFR 1.27 .

Items 12. to 17. below concern other document(s) or information included:

12. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ Other items or information: OTHER

09980824 .031402

JC10 Rec'd PCT/PTO 07 DEC 2001

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5) Unassigned 09/980824	INTERNATIONAL APPLICATION NO PCT/EP00/04848	ATTORNEY'S DOCKET NUMBER 016915-0252

18. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO $890.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) $740.00		
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))	$130.00	

Claims	Number Filed		Included in Basic Fee		Extra Claims		Rate		
Total Claims	9	-	20	=	0	×	$18.00	$0.00	
Independent Claims	1	-	3	=	0	×	$84.00	$0.00	
Multiple dependent claim(s) (if applicable)							$280.00		
TOTAL OF ABOVE CALCULATIONS =								$1020.00	
Reduction by ½ for filing by small entity, if applicable.								$510.00	
SUBTOTAL =								$510.00	
Processing fee of $130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f). +									
TOTAL NATIONAL FEE =								$510.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +									
TOTAL FEES ENCLOSED =								$510.00	
							Amount to be: refunded	$	
							charged	$	

a. ☒ A check in the amount of $510.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $0.00 to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Foley & Lardner
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5143

SIGNATURE 34371

NAME Glenn Law for RICHARD L. SCHWAAB

REGISTRATION NUMBER 25,479

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 016915-0252

In re patent application of
Gerd GEISSLINGER et al.
Serial No.: Unassigned
Filed: December 7, 2001
For: USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON β-GLUCURONIDASE IN HUMAN TISSUE

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

IN THE CLAIMS:

Please replace claims 3 through 9 as originally filed with the amended claims 3 through 9 as follows:

--3. (Amended) Use according to claim 1, characterised in that the R-enantiomers are used in pure form or, in comparison with the racemate, in enriched form.

4. (Amended) Use according to claim 1, characterised in that the glucuronidase inhibitor is used, with suitable pharmacologically compatible adjuvants, orally or parenterally in normally liberating or controlled liberating form.

5. (Amended) Use according to claim 1, characterised in that the glucuronidase inhibitor is used alone for the inhibition of β-glucuronidase in diseased tissue in order to prevent the progress of the disease, e.g. by inhibition of the tumour progression or the metastasis formation.

6. (Amended) Use according to claim 1, characterised in that the glucuronidase inhibitor is used for the stabilisation of metabolically-formed glucuronide conjugates of side-effect-rich active materials in order to reduce their side effects or to introduce a detoxification.

7. (Amended) Use according to claim 1, characterised in that the glucuronidase inhibitor is used combined with a glucuronide conjugate of an inflammation-inhibiting active material to be taken orally in order to protect this in the upper stomach-intestine tract against a cleavage and resorption and to activate in the deeper lying intestinal sections by cleavage for the intestinal local therapy.

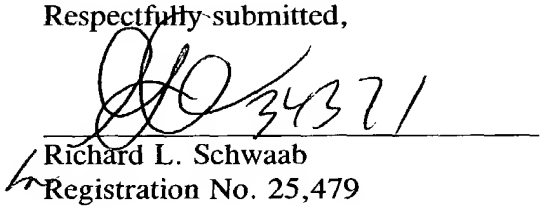
8. (Amended) Use according to claim 1 for the improvement of the tissue-specific therapy, characterised in that the glucuronidase inhibitor, in the case of combined use with a glucuronide prodrug, protects this against activation in healthy tissue in the case of maintenance of the activation in the target tissue.

9. (Amended) Use according to claim 1, characterised in that, besides the glucuronidase inhibitor and the glucuronide prodrug, there is used combined beta-glucuronidase bound to tissue-specific substances (e.g. antibodies, proteins, liposomes) in order to increase the activation of the prodrug in the target tissue and to protect the healthy tissue against the activation.--

REMARKS

Applicants respectfully request that the foregoing amendments to Claims 3 through 9 be entered in order to avoid this application incurring a surcharge for the presence of one or more multiple dependent claims. A marked-up version of the claims showing the changes made is attached.

Respectfully submitted,



December 7, 2001
Date

Richard L. Schwaab
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3000 K Street, N.W. Suite 500
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VERSIONS WITH MARKINGS TO SHOW CHANGES MADE

3. Use according to claim 1[or 2], characterised in that the R-enantiomers are used in pure form or, in comparison with the racemate, in enriched form.

4. Use according to claim 1[to 3], characterised in that the glucuronidase inhibitor is used, with suitable pharmacologically compatible adjuvants, orally or parenterally in normally liberating or controlled liberating form.

5. Use according to claim 1[to 4], characterised in that the glucuronidase inhibitor is used alone for the inhibition of β-glucuronidase in diseased tissue in order to prevent the progress of the disease, e.g. by inhibition of the tumour progression or the metastasis formation.

6. Use according to claim 1[to 4], characterised in that the glucuronidase inhibitor is used for the stabilisation of metabolically-formed glucuronide conjugates of side-effect-rich active materials in order to reduce their side effects or to introduce a detoxification.

7. Use according to claim 1[to 4], characterised in that the glucuronidase inhibitor is used combined with a glucuronide conjugate of an inflammation-inhibiting active material to be taken orally in order to protect this in the upper stomach-intestine tract against a cleavage and resorption and to activate in the deeper lying intestinal sections by cleavage for the intestinal local therapy.

8. Use according to claim 1[to 4] for the improvement of the tissue-specific therapy, characterised in that the glucuronidase inhibitor, in the case of combined use with a glucuronide prodrug, protects this against activation in healthy tissue in the case of maintenance of the activation in the target tissue.

9. Use according to claim 1[to 4 and 8], characterised in that, besides the glucuronidase inhibitor and the glucuronide prodrug, there is used combined beta-

glucuronidase bound to tissue-specific substances (e.g. antibodies, proteins, liposomes) in order to increase the activation of the prodrug in the target tissue and to protect the healthy tissue against the activation

Applicant or Patentee: Gerd GEISSLINGER et al.
Serial or Patent No.: Unassigned Atty. Dkt. No. 016915-0252
Filed or Issued:
For: USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON BETA-GLUCURONIDASE IN HUMAN TISSUE

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.27) — SMALL BUSINESS CONCERN

I hereby declare that I am

( ) the owner of the small business concern identified below:
(X) an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: PAZ ARZNEIMITTEL-ENTWICKLUNGS GESELLSCHAFT MBH

ADDRESS OF CONCERN: In der Schildwacht 13
D-65933 Frankfurt am Main
Federal Republic of Germany

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18 and reproduced in 37 CFR 1.27, for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON BETA-GLUCURONIDASE IN HUMAN TISSUE by Gerd GEISSLINGER et al., described in

(X) the specification filed herewith
( ) application serial no. _____, filed _____
( ) patent no._____ , issued _____

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no

Serial No.: ATTORNEY DOCKET NO.

rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.27(a)(1) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.27(a)(2) or a nonprofit organization under 37 CFR 1.27(a)(3). * NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities: (37 CFR 1.27)

NAME: _____
ADDRESS: _____
( ) INDIVIDUAL( ) SMALL BUSINESS CONCERN( ) NONPROFIT CORPORATION

NAME: _____
ADDRESS: _____
( ) INDIVIDUAL( ) SMALL BUSINESS CONCERN( ) NONPROFIT CORPORATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.27.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Dr. Otto Schuster
TITLE OF PERSON OTHER THAN OWNER: _____
ADDRESS OF PERSON SIGNING: 65812 Bad Soden, Kelkheimer Str. 69, Germany
SIGNATURE: Otto Schuster DATE: 4 Dec. 2001

41PRTS

Use of verapamil and verapamil derivatives for the preparation of pharmaceuticals with β-glucuronidase-inhibiting action in human tissue

The subject of the present invention is the use of verapamil or verapamil derivatives in pharmaceuticals for the inhibition of the enzyme beta-glucuronidase in human tissue with the object directly to achieve therapeutic effects or to improve its therapeutic breadth by combined use together with glucuronidated or glucuronidatable active materials.

The conjugation of endogenic or exogenic substances with glucuronic acid is an important metabolic reaction in humans and animals. Glucuronic acid can be conjugated with the most varied substances, e.g. pharmaceutically active materials and their metabolites. The conjugation reaction takes place by transfer of activated glucuronic acid (UDP-glucuronic acid) to the substrate by means of the enzyme glucuronyl transferase. In general, the organism uses the conjugation reaction for detoxication since glucuronic acid conjugates are usually less toxic and, on the basis of their good water solubility, are easily excreted via the kidneys or the gall secretions via the intestines. A conjugation can also take place in non-enzymatic ways by chemical synthesis.

The glucuronic acid conjugates can, however, also be cleaved by catalytic action of glucuronidases into glucuronic acid and into the starting product. The cleavage of glucuronides frequently takes place after excretion thereof via the bile in deeper lying small intestine sections or in the large intestine. The thereby resulting starting substances can again be resorbed and this become renewed active in the organism. This process, designated as enterohepatic circulation, can prolong the desired action of substances but can also increase the toxic actions of poisonous substances.

By medicamentous regulation of the beta-glucuronidase activity in the various tissues, new therapy concepts are opened up.

Use of glucuronidase inhibitors in cancer therapy.

A peculiarity of cancer tissues is their high concentration of beta-glucuronidases or an extremely high glucuronidase activity. Closely associated with the increased glucuronidase activity is the tendency to form certain tumour metastases. By general administration of a beta-glucuronidase inhibitor, in the case of tumours which, on the basis of the increased beta-glucuronidase activity, tend to the progression and metastasis formation, the tumour spreading out is reduced via the inhibition of the tumour glucuronidase. Saccharo-1,4-lactone, 2-acetamidoglycal and heparin derivatives were tested for this purpose /Bernacki R.J., Cancer Metastasis Rev., (1985) 4: 81 - 101;Nakajima M., Journal of Cellular Biochemistry (1988) 36: 157 - 167; Niwa T., Journal of Biochemistry (1972) 72: 207 - 217. In most recent times, selective glucuronidase inhibitors have been synthesised (Bosslet K., EP 0822192).

Besides the general use for the therapy, glucuronidase inhibitors can also be used supportingly in the chemotherapy of cancer patients for the increasing of the desired effect in the case of simultaneous reduction of the undesired actions.

The chemotherapy causes an extraordinary physical and psychic stressing of the cancer patient. Glucuronidase inhibitors can ameliorate negative actions of the chemotherapy and simultaneously increase the effectiveness of the therapy. For this purpose, the following starting points present themselves.

Chemotherapeutics are, inter alia, also excreted via their glucuronides via small intestines. Due to the actions of the there-present glucuronidases, there takes place a cleavage of these glucuronides and liberation

of the active cell-toxic substances which damage the intestinal tissue present in continuous cell division and regeneration. For the patient, there result therefrom nausea, vomiting and diarrhoea, combined with a fluid and weight loss.

Beta-glucuronidase inhibitors can protect the intestines against toxic products from cytostatic glucuronides. Thus,e.g. the intestinal toxicity of the anti-tumour agent irinotectan hydrochloride can be minimised by preventative administration of the beta-glucuronidase inhibitor baicalin. The patients are thus protected against a massive diarrhoea and the fluid losses involved therewith /Takasuna K. Jpn. Cancer Res. (1995) 86: 978 - 84;: Kamataki T. U.S. Pat. 5,447,719).

Considerations exist of using the cleavage of glucuronides in certain tissues in order to liberate the active substances from inactive precursors of active medicaments (prodrugs). Due to the preferred liberation in the diseased target tissues, via the increased substance concentration, there can be achieved a more or less local action in the case of low systemic action /Sperker B., Clin. Pharmakinet. (1997) 33: 18 - 317. This therapy possibility would be of interest above all in the case of the use of side effect-rich substances in tumour therapy because the desired cytotoxic properties of chemotherapeutics can be concentrated on the tumour tissues. The tumour progression and the metastasis formation is frequently bound up with an increased glucuronidase activity. In necrotic tumour regions, an increased glucuronidase activity is present in the extracellular space whereas in the healthy tissue the glucuronidase activity is substantially intracellular localised. A pH value in the tumour displaced towards acid can again increase the activity of the beta-glucuronidase. These physiological conditions offer starting points for the application of glucuronic acid conjugates with chemotherapeutics to tumour patients for the local

liberation of the active substrate after cleavage by the locally increased glucuronidase activity /Sperrker B., Clin. Pharmacokinet. (1997) 33: 18 - 317. The local action could be strengthened by simultaneous administration of a glucuronide prodrug and of a tumour-specific antibody which is covalently bound with beta-glucuronidase (antibody-directed prodrug therapy = ADEPT)/Sperker B., Clin. Pharmacokinet. (1997) 33: 18 - 317.

The increased tumour selectivity of glucuronide prodrugs leads to correspondingly higher active material levels in the tumour and sumultaneously to lower active material concentrations in healthy tissue regions, i.e. the effectivenesses and compatabilities of the chemotherapeutics are increased.

Known examples are doxorubicin glucuronide prodrugs which, in comparison with the free doxorubicin, make possible in tumour tissues an about 10 times higher doxorubicin level but, at the same time, protects healthy tissue with a lower concentration so that e.g. the typical cardiotoxic property of doxorubicin only plays a subsidiary role /Bosslet K., Cell Biophys. (1994) 24-25; 51-63; Bosslet K., Cancer Res. (1994) 54: 2151-9; Bosslet K., Cancer Res. (1998): 1195 - 201; Murdter, T.E., Cancer Res. (1997) 57: 2440-57.

None of these investigations has hitherto lead to therapeutically usable results, i.e. utilisable medicaments.

Description of the invention

The invention has set itself the task of finding glucuronidase inhibitors which are otherwise pharmacologically not or only little effective, i.e. display few side reactions, in order to use these as medicaments in the above-described uses alone or in combination with other medicaments for the increasing of the therapeutic breadth.

This task is solved by the features of the main claim and promoted by the features of the subsidiary claim.

It is known that verapamil inhibits the activity of bacterial beta-glucuronidase /E. coli/ to a considerable extent (B. Sperker et al., Eur. J. Clin. Pharm. (1999), Vol. 55, A, 16) but does not inhibit the glucuronidase in the intestinal tissue of rats (mammals) in contradistinction to known glucuronidase inhibitors, such as D-saccharic acid 1,4-lactose, which, in the case of the rat enzyme, inhibits 30 times more strongly than the enzyme from E. coli.

Surprisingly, it has now been found that verapamil exerts a strong inhibiting action on the β-glucuronidase occurring in the human tissues. The inhibition takes place in the case of an administration of 1 - 10 mg per kg body weight and day to an equal extent by the racemic mixture and the pure enantiomers. It is known that the diverse actions of verapamil, known as calcium antagonist, on the heart and vascular system essentially come from the S-enantiomer /Mickisch G.H., J. Cancer Res. Clin. Oncol. (1995) 121 (Suppl. 3): R11 - R16/. Thus, in the case of the scarcely cardioactively effective R-enantiomer of verapamil or verapamil derivatives, the desired inhibiting effects on the beta-glucuronidase activity are achieved without the pharmacological actions known for verapamil occurring as undesired side effect.

In particular, the adjuvant oral administration of retarded medicaments of verapamil or its derivatives is intended for uses which, over comparatively long periods of time, are to protect the intestines against the toxic cleavage products for less toxic β-glucuronides. In the case of adjuvant administration in cancer therapy, the thereby also occurring systemic distribution of the inhibitors of the verapamil type is no disadvantage. It is known that verapamil favourably influences the treatment

of chemotherapy-resistant cancer cells /Volm M., Anticancer Res. 18 (C4): 2905 - 17; Wainer I.W., Ann. Oncol. (1993), 4 (Suppl. 2): 7 - 137. Various mechanisms of the manner of working are thereby discussed, whereby verapamil suppresses the active passing out of the chemotherapeutic from the cancer cells /Simpson W.G., Cell Calcium (1985) 6: 449 - 677 or perhaps prevents the expression of multidrug resistance genes /Ling V., Cancer Chemother. Pharmacol. (1997) 40 (Suppl.): S3 - S8; Mickisch G.H., J. Cancer Res. Clin. Oncol (1995) 121 (Suppl. 3): R11 - R167. A participation of β-glucuronidases is not given the case of these mechanisms.

Glucuronidase inhibitors of the verapamil type can also be used supportingly in chemotherapy together with novel glucuronide prodrug chemotherapeutics. The therapy supporting with glucuronidase inhibitors of the verapamil type comprises the protection of the healthy tissue against the actions of these chemotherapeutics, especially against the actions of higher local concentrations at injection points or other places of introduction.

The verapamil administration and dosing takes place in such a way that locally at the infusion entrance the healthy tissue is protected, i.e. the glucuronidases are there inhbited but, after the systemic mixing up, no deactivation of the tumour glucuronidases takes place in the tumour tissue.

Physiologically less stable glucuronide prodrugs can pharmaceutically be so stabilised by addition of the glucuronidase inhibitor verapamil that only after the systemic mixing up in the organism does the cleavage preferably take place in the target tissue.

In the case of administration of biologically-inactive glucuronide prodrugs, together with beta-glucuronidase inhibitor, the cleavage into the effective substrate is delayed so that, in the case of prodrugs with long elimination half value time, the systemic availability

is prolonged. Correspondingly, the dose can be reduced and the dosaging interval lengthened.

In the case of the tumour-specific prodrug therapy, by additional administration of a cell membrane-permeable beta-glucuronidase inhibitor, such as verapamil, the therapeutic breadth is thereby increased that the substantially intracellularly present beta-glucuronidase is inhibited in healthy tissue and a pharmacological action is thereby hindered. In the tumour tissue, due to the physiological or due to the glucuronidase concentration increased by ADEPT therapy, the effective substrate is, as previously, formed in the case of suitable choice of dose.

The inhibiting action on the beta-glucuronidase activity claimed in the invention is verified in the results set out in the following.

Investigations of the lowering of human β-glucuronidase activity by verapamil, its metabolites and gallopamil.

The calcium antagonist verapamil (not only racemate but also both enantiomers), its metabolites and the derivative gallopamil are in the position to lower the activity of the human β-glucuronidase.

A direct inhibition of the β-glucuronidase activity could be shown in experiments with human liver homogenates. For this purpose, homogenates of various liver samples were incubated with 2.5 mM 4-methyl-belliferyl-β-D-glucuronide (MUG) and analysed by means of HPLC. The concentrations of the liberated 4-methylumbelliferone is a measure of the activity of the β-glucuronidase. In the case of homogenates which, in addition to MUG, also received 100 µM verapamil (racemate), the activity was reduced significantly by about 25%, in comparison with the control samples (Fig. 1).

Parallel bring about verapamil, the metabolite norverapamil, D702, D 703 and gallopamil in the human hepatoma cell line HepG2 after 48 h incubation a reduction of the β-glucuronidase activity to 50 - 65% which is to

be attributed to a reduced expression of the enzyme. This reduction of the activity is concentration dependent (Fig. 2).

The reduction of the β-glucuronidase activity could be observed equally strongly with verapamil racemate and with R- and S-verapamil. The metabolites norverapamil, D 702 and D 703 show a comparable influence on the activity of the β-glucuronidase in HepG2 cells. The incubation with D 617, a further metabolite, only brings about a lowering of the activity by 12% which, however, is not statistically significant. Gallopamil brings about an effect comparable to verapamil (Fig. 3).

Example 1

Inhibition of the activity of human liver β-glucuronidase by verapamil (Fig. 1).

Human liver homogenates were incubated with the enzyme substrate 4-methylbelliferyl-β-D-glucuronide (1 h, 37°C). 100 µM verapamil or DMSO (control) were added to the reaction mixture. The liberation of 4-methylumbelliferone was measured by means of HPLC analysis (* significant difference to the control; $p < 0.001$; n = 3 independent experiments).

Example 2

Concentration dependency of the verapamil action in the human hepatoma cell line HepG2 (Fig. 2).

HepG2 cells were incubated for 48 h at 37°C with the concentrations of verapamil given in Fig. 2. After lysis of the cells, in each case 2.25 µg of cellular protein were incubated (2 h, 37°C) with the glucuronidase substrate 4-methylumbelliferyl-β-D-glucuronide and the concentration of the liberated 4-methylumebelliferone measured by mean of HPLC (* significant difference to the control, $p < 0.05$).

Example 3

Lowering of the β-glucuronidase activity in HepG2 cells by incubation with verapamil, verapamil metabolites and gallopamil (Fig. 3).

HepG2 cells were incubated for 48 h at 37°C with 100 µM verapamil (Vera), in each case 100 µM D617, D702, D703, 30 µM norverapamil (Nor) or 100 µM gallopamil (Gallo). After lysis of the cells, the β-glucuronidase activity was determined by means of 4-methylumbelliferyl-β-D-glucuronide cleavage (significant difference to the control, * $P < 0.01$, ** $p < 0.001$, n = 3 independent experiments).

Example 4

Lowering of the beta-glucuronidase expression by verapamil in the human hepatoma cell line HepG2 (Fig. 4).

HepG2 cells were incubated 48 h at 37°C with 100 µM verapamil or DMSO (control). After lysis of the cells, 50 µg cellular protein were separated off by means of SDS page, transferred to nitrocellulose and subsequently incubated with the monoclonal antibody 2156/42. The band intensity was determined densitometrically (DE = densitometric units; * significant difference to the control, $p < 0.05$; n = 3 independent experiments).

Inhibition of the glucuronidases in the rat intestine by verapamil (comparison)

In a study with Sprague-Dawley rats, the absorption of orally administered morphine-6-glucuronide (M6G) to two groups (group 1: n = 5, without verapamil administration; group 2: n = 4 previous verapamil administration) was investigated. The study was carried out with rats since these cannot form M6G from morphine (Aasmundstad T.A., Biochem. Pharmacol. (1993) 46: 961-968) so that the M6G measured in the plasma originated from the absorption of the orally administered M6G.

Whereas the previous administration of verapamil had no influence on the height of the plasma concentration of M6G or its variation in time, the concentrations of morphine and M3G in the case of previous verapamil administration (group 2) were distinctly smaller than in the case of the group without verapamil (group 1) (Fig. 5).

The absent influence on the height of the plasma concentration of M6G or its variation in time makes it improbable that the reduction of the morphine and M3G absorption depends upon an inhibition of the intestinal mobility /Shah M.H., J. Pharm. Pharmacol. (1987) 39: 1037 - 1038; Krevsky B., Dig. Dis. Sci. (1992) 37: 919 - 924/. It is known that M6G inhibits the intestinal motility with the same potency as morphine /Schmidt N., Eur. J. Pharmacol. (1994) 255: 245 - 237/. An increase of this inhibition by verapamil /Shah M.H., J. Pharm. Pharmacol. (1987) 39, 1037-1038/ acts with all probability on M6G and morphine to the same extent. On the other hand, only the plasma level of morphine or M3G but not of M6G were reduced, i.e. the cleavage of M6G available after oral administration to morphine is thus inhibited. Therefrom result lower morphine and, as a result, M3G plasma levels since the greater part of the absorbed morphine is metabolised by glucuronyl transferases to M3G. The carrying out of the experiments is described in Example 5.

Example 5

Plasma concentration time progression of morphine-6-glucuronide (M6G), morphine and morphine-3-glucuronide (M3G) after oral administration to Sprague-Dawley rats of M6G with or without previous oral administration of verapamil (Fig. 5)

The investigation was carried out on 9 male Sprague-Dawley rats. The rats were divided into 2 groups: group 1 (5 animals, weight: 258.6 ± 31.2 g) received only 62.5 mg/kg morphine-6-glucuronide (M6G) administered orally. Group 2 (4 animals, weight 272 ± 8 g) received, 15 minutes before M6G administration (62.5 mg/kg orally), 70 mg/kg verapamil orally administered. The groups did not differ significantly from one another with regard to their weight (t-test: t = -0.923, p = 0.401; confidence interval for difference group 1 - group 2: -51.6 to 24.8 g)

M6G and verapamil were dissolved in Ringer lactate and subsequently mixed with tylose mucilage. To each rat were administered orally 62.5 mg M6G per kg body weight in tylose mucilage. 15 min before administration of M6G, 4 rats received 70 mg verapamil per kg body weight orally administered in tylose mucilage.

For the determination of the plasma concentrations of M6G, morphine and M3G, in the case of each rat 6 blood samples were taken (each about 200 µl) at the following times: before the administration of M6G, as well as 1, 2, 4, 6 and 8 hours after M6G administration. The blood samples were transferred into heparinised EDTA synthetic resin test tubes and immediately centrifuged. Until analysis, the prepared blood samples were stored at -20°C. The concentration of M6G, morphine and morphine-3-glucuronide (M3G) were determined by means of HPLC (cf. Hartley R., Biomed. Chromatog. (1993) 7: 34 - 37). The detection limit lay for all three substances at 10 ng/ml, i.e. 35.05 nmol/l for morphine and 22.45 nmol/l for the morphine glucuronides. In the whole calibration range, the variation coefficient in the whole calibration range (10 - 500 ng/ml) lay below 11%.

Inhibition of microbial beta-glucuronidase by verapamil

From Example 5 is to be seen that a cleavage of glucuronides (M6G) takes place in the intestines of the rat. It is not to be seen whether beta-glucuronidases of the rat and/or microbial beta-glucuronidases (e.g. E. coli) are responsible for this cleavage.

In order to clarify this question, beta-glucuronidases from rat intestine homogenates and from E. coli were incubated with verapamil or D-glucaric acid-1,4-lactone in the presence of 4-methylumbelliferyl-β-D-glucuronide (MUG). The cleavage of the 4-methylumbelliferyl-β-D-glucuronide is a measure for the activity of the beta-glucuronidase. As is to be expected, D-glucaric acid-1,4-lactone inhibits not only the beta-glucuronidase activity of

the rat intestine homogenates but also the E-coli beta-glucuronidase (Fig. 6A and B). Surprisingly, the bacterial enzyme was clearly inhibited by verapamil (IC_{50} = 30 µM), whereas the rat beta-glucuronidase is not measurably influenced by verapamil (Fig. 6A and B).

The carrying out of the experiment is described in Example 6.

Example 6

Inhibition of 4-methylumbelliferyl-β-D-glucuronide (MUG) cleavage by verapamil and D-glucaric acid-1,4-lactone (Fig. 6).

Deep frozen tissue powder of a rat mucosa (duodenum and jejunum) was suspended in 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM pefabloc® (firm Roth, Karlsruhe, Germany). The protein concentration was determined according to the method of Lowry [Lowry O.H., J. Biol. Chem. (1951) 193: 265 - 275]. The incubation and analysis took place according to: [Sperker B., J. Pharmacol. Exp. Ther. (1997) 281: 914 - 920]. 50 µl incubation mixture contained 2.25 µg rat protein homogenate or 110 pg (0.001 units) purified E. coli beta-glucuronidase (firm Sigma, Deisenhofen, Germany). The test buffer contained 0.2 mM MUG (firm Sigma, Deisenhofen, Germany).

The incubation mixtures were mixed at 37°C with verapamil or D-glucaric acid-1,4-lactone. After 10 minutes, the MUG buffer was added. After 1 hour at 37°C, the enzymatic reaction was stopped by addition of 150 µl 200 mM sodium carbonate solution. After centrifuging (5 min., 13,000 r.p.m.), the supernatants were analysed by means of HPLC (fluorescence: absorption 355 nm, emission 460 nm). The enzyme activity was correlated with the liberation of 4-methylumbelliferone (MU). The experiments were carried out at the corresponding optima of the beta-glucuronidases (pH 7.0 E. coli or pH 5.0 rat). The results of Fig. 6 show that verapamil is not able

to inhibit the glucuronidase of the rat but is a good inhibitor for the bacterial glucuronidase from E. coli.

On the other hand, the known inhibitor D-glucaric acid 1,4-lactone inhibits both enzymes equally well.

Patent Claims

1. Use of verapamil or verapamil derivatives for the inhibition of human tissue glucuronidase.

2. Use according to claim 1 characterised in that, as verapamil derivatives, there are used its R-enantiomer, metabolites of verapamil, gallopamil or chemically substituted derivatives of verapamil, gallopamil and its metabolites or its salts with pharmacologically compatible acids.

3. Use according to claim 1 or 2, characterised in that the R-enantiomers are used in pure form or, in comparison with the racemate, in enriched form.

4. Use according to claim 1 to 3, characterised in that the glucuronidase inhibitor is used, with suitable pharmacologically compatible adjuvants, orally or parenterally in normally liberating or controlled liberating form.

5. Use according to claim 1 to 4, characterised in that the glucuronidase inhibitor is used alone for the inhibition of β-glucuronidase in diseased tissue in order to prevent the progress of the disease, e.g. by inhibition of the tumour progression or the metastasis formation.

6. Use according to claim 1 to 4, characterised in that the glucuronidase inhibitor is used for the stabilisation of metabolically-formed glucuronide conjugates of side-effect-rich active materials in order to reduce their side effects or to introduce a detoxification.

7. Use according to claim 1 to 4, characterised in that the glucuronidase inhibitor is used combined with a glucuronide conjugate of an inflammation-inhibiting active material to be taken orally in order to protect this in the upper stomach- intestine tract against a cleavage and resorption and to activate in the deeper lying intestinal sections by cleavage for the intestinal local therapy.

8. Use according to claim 1 to 4 for the improvement of the tissue-specific therapy, characterised in that the glucuronidase inhibitor, in the case of combined use with a glucuronide prodrug, protects this against activation in healthy tissue in the case of maintenance of the activation in the target tissue.

9. Use according to claim 1 to 4 and 8, characterised in that, besides the glucuronidase inhibitor and the glucuronide prodrug, there is used combined beta-glucuronidase bound to tissue-specific substances (e.g. antibodies, proteins, liposomes) in order to increase the activation of the prodrug in the target tissue and to protect the healthy tissue against the activation.

Summary

The present invention concerns the use of verapamil or verapamil derivatives for the production of medicaments with action inhibiting glucuronidase in human tissue.

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— *Vor Ablauf der für Änderungen der Ansprüche geltenden Frist; Veröffentlichung wird wiederholt, falls Änderungen eintreffen.*

Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

WO 00/74670 A1

(54) Title: USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON β-GLUCURONIDASE IN HUMAN TISSUE

(54) Bezeichnung: VERWENDUNG VON VERAPAMIL UND VERAPAMILDERIVATEN ZUR HERSTELLUNG VON ARZNEIMITTELN MIT β-GLUCURONIDASE IM HUMANEN GEWEBE HEMMENDER WIRKUNG

(57) Abstract: The invention relates to the use of verapamil or verapamil derivatives for producing medicaments which have an inhibiting effect on β-glucuronidase in human tissue.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft die Verwendung von Verapamil oder Verapamilderivaten zur Herstellung von Arzneimitteln mit Glucuronidase im humanen Gewebe hemmender Wirkung.

Title: USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON BETA-GLUCURONIDASE IN HUMAN TISSUE
Inventor(s): Gerd GEISSLINGER et al.
DOCKET NO.: 016915-0252

Fig. 1

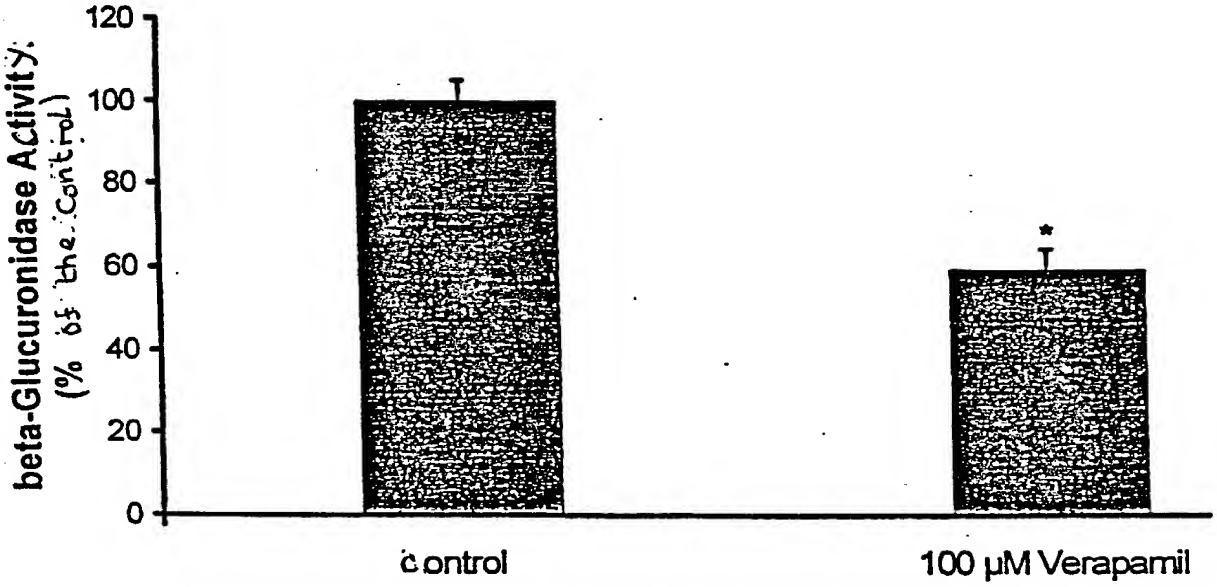
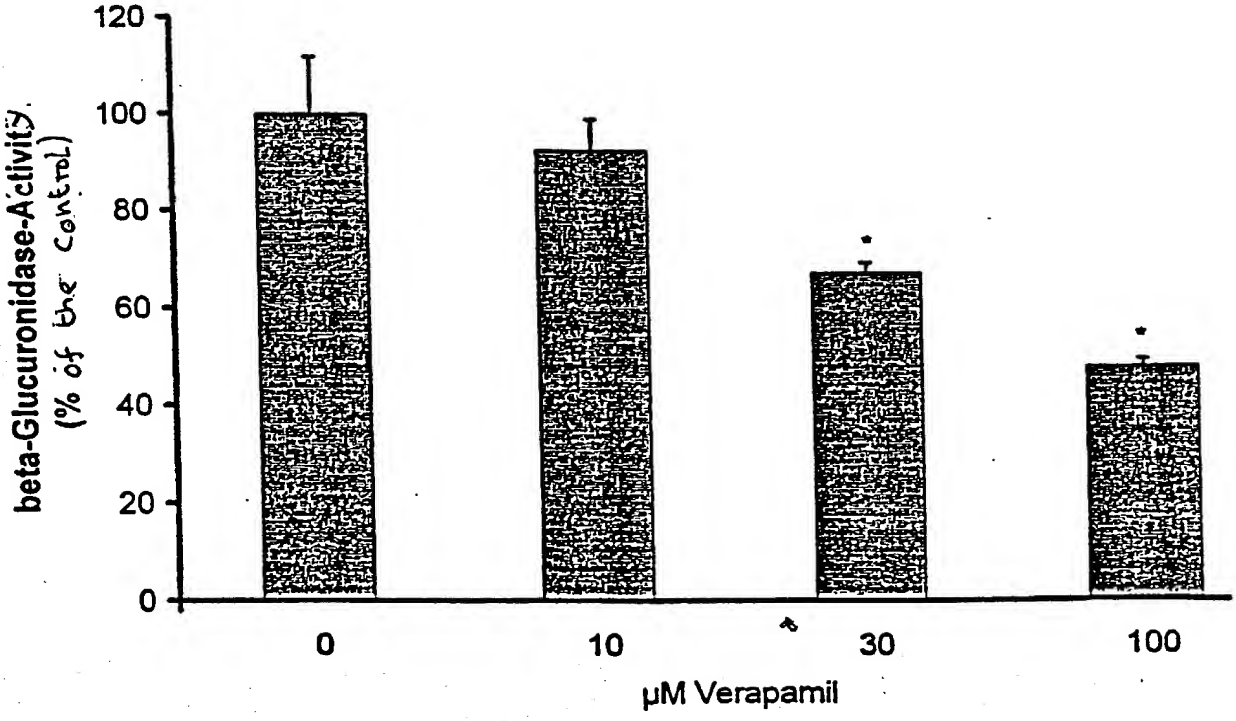


Fig. 2



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Title: USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON BETA-GLUCURONIDASE IN HUMAN TISSUE
Inventor(s): Gerd GEISSLINGER et al.
DOCKET NO.: 016915-0252

Fig. 3

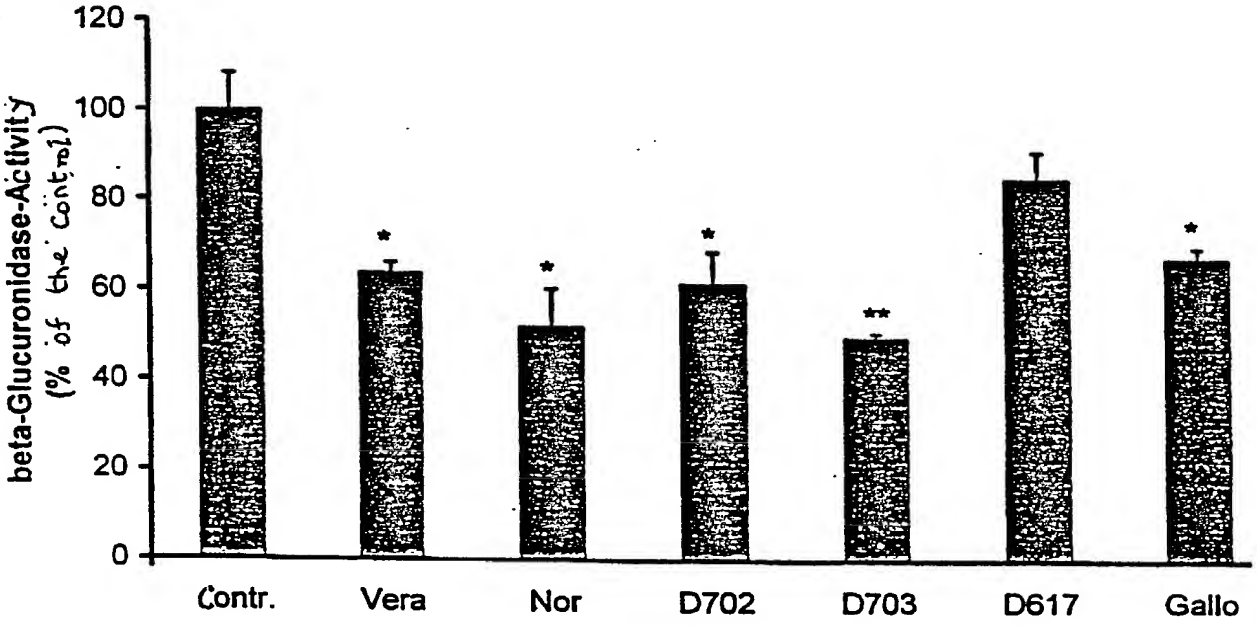


Fig. 4

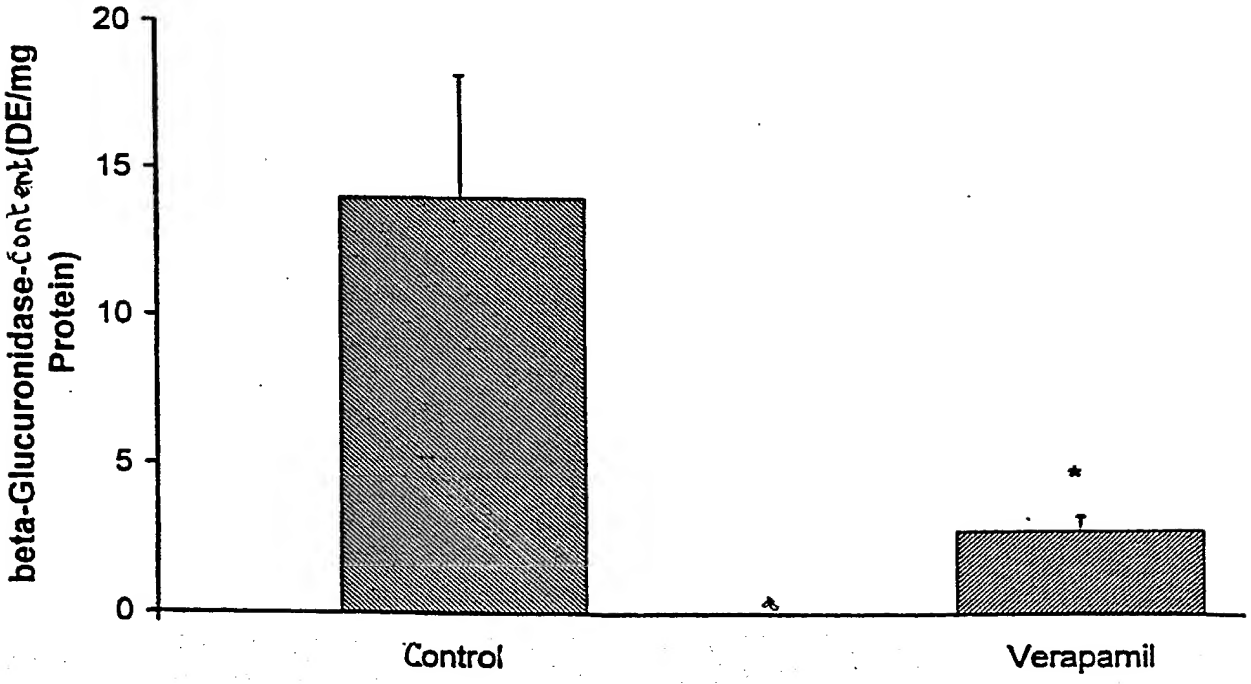
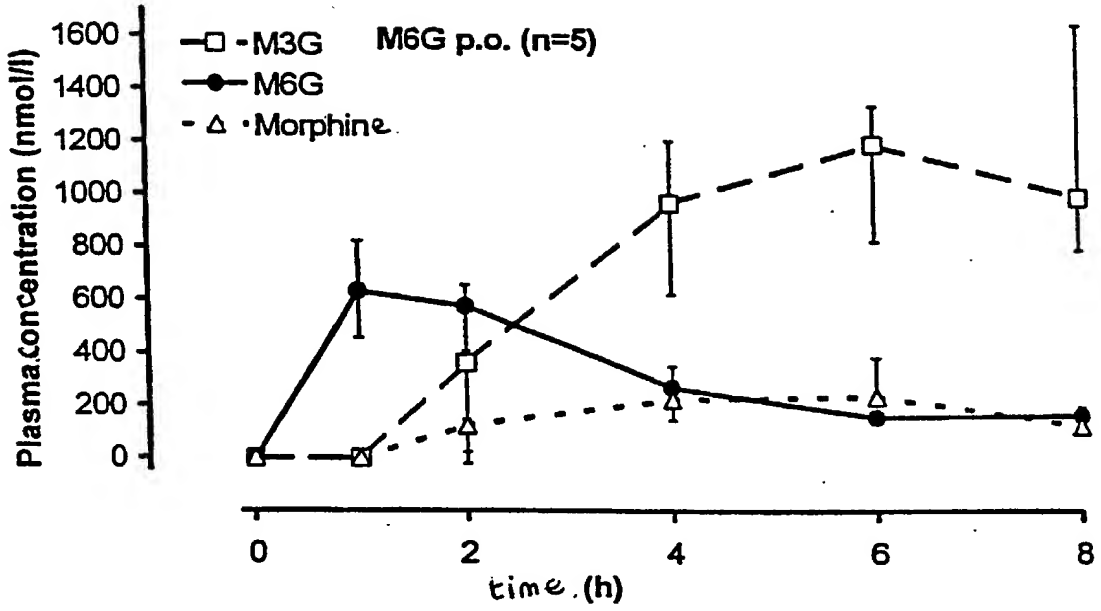


Fig. 5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M6G
Morphine
Plasma concentration (nmol/l)
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1200
1000
800
600
400
200
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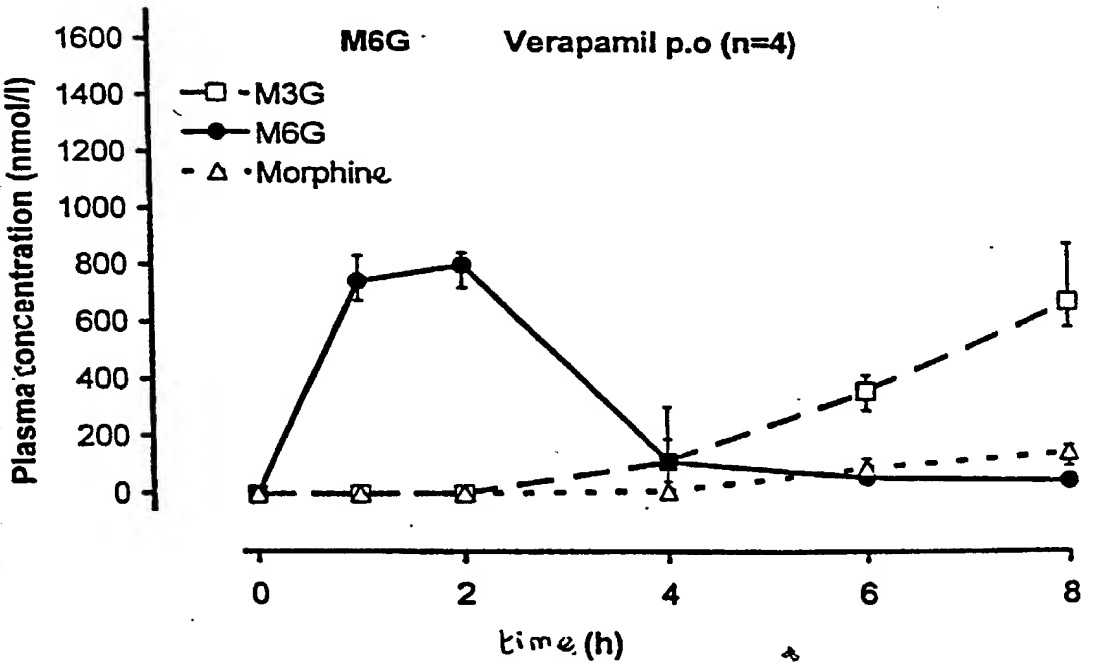
0
2
4
6
8
time (h)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Verapamil p.o (n=4)
M3G
M6G
Morphine
Plasma concentration (nmol/l)
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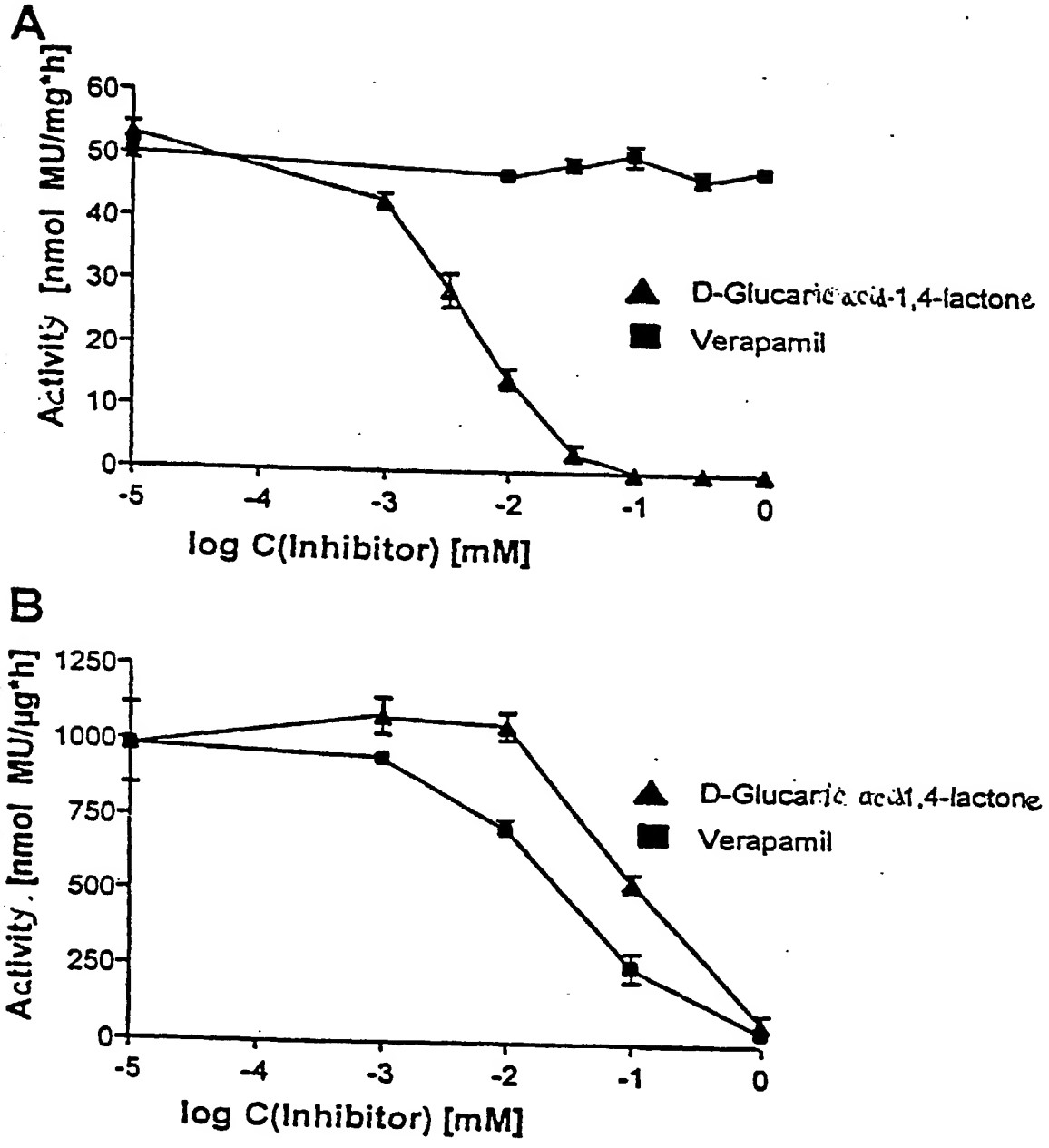
1200
1000
800
600
400
200
0
0
2
4
6
8
time (h)

Fig. 6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50
40
30
20
10
0
-5
-4
-3
-2
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0
log C(Inhibitor) [mM]
D-Glucaric acid-1,4-lactone
Verapamil
B
Activity [nmol MU/µg*h]
1250
1000
750
500
250
0
-5
-4
-3
-2
-1
0
log C(Inhibitor) [mM]
D-Glucaric acid-1,4-lactone
Verapamil

09980024 PRT 4732 PCT/US

Atty. Dkt. No. 016915-0252

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

USE OF VERAPAMIL AND VERAPAMIL DERIVATIVES FOR PRODUCING MEDICAMENTS WITH AN INHIBITING EFFECT ON BETA-GLUCURONIDASE IN HUMAN TISSUE

(Attorney Docket No. 016915-0252)

the specification of which (check one)

____ is attached hereto.

__X__ was filed on May 27, 2000 as United States Application Number or PCT International Application Number PCT/EP00/04848 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

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THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Atty. Dkt. No. 016915-0252

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
199 25 810.4	Federal Republic of Germany	June 7, 1999	YES	

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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